I hereby certify that this correspondence is being electronically transmitted to the United States Patent and Trademark Office on 21 August 2009.

Jeff Lloyd, Patent Attorney

REQUEST FOR CERTIFICATE
OF CORRECTION UNDER
37 CFR 1.322 and 1.323
Docket No. UF.289C2
Patent No. 7,494,955 B2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Raghavan Charudattan, Matthew Scott Pettersen, Ernest Hiebert
Issued	:	February 24, 2009
Patent No.	:	7,494,955 B2
Conf. No.	:	7738
For	:	Use of Tobacco Mild Green Mosaic Virus (TMGMV) Mediated Lethal Hypersensitive Response (HR) as a Novel Method of Weed Control

ATTN: CERTIFICATE OF CORRECTION BRANCH
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

<u>REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322 (OFFICE MISTAKE) AND UNDER 37 CFR 1.323 (APPLICANTS' MISTAKE)</u>

Sir:

A Certificate of Correction for the patent identified above has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 1, Line 54: "The a typical"	Page 2, Line 12: --The atypical--
Column 9, Line 28: "*C. annuum* L. (Jalapeno)"	Page 15, Table 4: --*C. annuum* L. (Jalapeño)--

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Patent Reads:	**Application Should Read:**
Column 9, Line 57: "indirect ELSIA"	Page 15, Footnote *a*: --indirect ELISA--
Patent Reads:	**Application Reads:**
Column 10, Lines 4-5: "Jalapefño)"	Page 16, Line 6: --Jalapeño)--
Column 15, Line 50: "Chisholn et al."	Page 26, Line 9: --Chisholm et al.--

A true and correct copy of pages 2, 15, 16, and 26 of the specification as filed which supports Applicants assertion of errors on the part of the Patent Office accompanies this Certificate of Correction.

The Commissioner is authorized to charge any additional fees as required under 37 CFR 1.20(a) to Deposit Account No. 19-0065.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

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JL/jlr
Attachments: Copy of pages 2, 15, 16, and 26 of the specification
Certificate of Correction

Summary of the Invention

All references cited herein are incorporated by reference in their entirety, to the extent not inconsistent with the explicit teachings set forth herein.

As an alternative to chemical herbicides, we searched for a suitable pathogen of tropical soda apple (TSA) for development as a bioherbicide and have discovered that Tobacco mild green mosaic virus (TMGMV) induces a lethal, systemic, hypersensitive response in TSA. TMGMV is a member of the tobamoviruses, which consist of mechanically transmitted, rod-shaped, RNA viruses that are strictly plant pathogens. The type species of *Tobamovirus* is Tobacco mosaic virus U1 (TMV U1), a widely distributed plant virus. Unlike TMGMV, TMV U1 and Tomato mosaic virus (ToMV, another *Tobamovirus* species), caused only mild, nonlethal mosaic or mottling of the TSA leaves. The atypical lethal effect of TMGMV on TSA was unexpected and is previously unknown. Also unknown was the feasibility to use TMGMV as a biocontrol for TSA.

Tropical soda apple serves as a host for TMV U1, ToMV, and TMGMV. In contrast to the mild, systemic mosaic symptoms caused by TMV U1 and ToMV, TMGMV causes rapid death of TSA. This death occurs due to a massive, systemic, hypersensitive plant response to infection by the virus. Both serological and molecular evidence confirm that TMGMV is responsible for the rapid and high rate of mortality on TSA. The age of TSA at the time of TMGMV inoculation does not affect the mortality rates, but the first expression of symptoms and first plant mortality are slightly delayed in older plants as compared to younger plants. Thus, the ability to control TSA by TMGMV is not limited by plant age. Temperature is usually not a limiting factor, although disease development will be slowed or prevented if the inoculated TSA plants are maintained continuously at 32°C (or presumably at higher temperatures). However, under normal field conditions, a diurnal temperature fluctuation will occur and as our results indicate, TMGMV kills TSA plants under the diurnal cycle of 32/22°C temperatures. To avoid possible adverse effects of high temperatures according to the subject invention, the TMGMV is preferably used in the field during the cooler months of spring and fall.

Table 4

Reaction of Some *Solanum* Species to TMGMV

	Absorbance values (405 nm)[a]		Host Reaction[b]
Host species	Treated	Control	
C. annuum L. (Jalapeño)	1.09	0.303	SHR +
C. annuum cv. California Wonder			SHR +
L. esculentum Mill. cv. Better Boy			
S. acerifolium Dunal	0.398	0.277	
S. aculeatissimum Jacq.	0.186	0.18	
S. aethiopicum L.	0.08	0.09	
S. americanum Mill.	0.02	0.07	LHR
S. anguivi Lam.	0.02	0.03	LHR
S. atropurpureum Schrank			
S. aviculare G. Forst.			
S. capsicoides All.			
S. caripense Dunal			
S. ciliatum Lam.			
S. elaeagnifolium Cav.	0.02	0.03	
S. ferox auct. = *S. lasiocarpum* Dunal			
S. gilo Raddi = *S. aethiopicum* L.			LHR
S. incanum L.			
S. linnaeanum Hepper & P.M.L. Jaeger			
S. macrocarpon L.			M+
S. *mammosum* L.			
S. melongena L.			
S. nigrum L. var. *villosum* L. = *S. villosum* Mill.	0.44	0.06	+NS
S. nodiflorum Jacq. = *S. americanum* Mill.	0.14	0.09	LHR
S. pseudocapsicum L.	0.015	0.02	LHR
S. rostratum Dunal	0.19	0.03	+NS
S. sessiliflorum Dunal			LHR
S. sisymbriifolium Lam.	0.05	0.03	
S. spinosissium Lodd. ex G. Don, nom. nud.	0.2	0.01	M+
S. stramoniifolium Jacq.	0.06	0.04	
S. suaveolens Kunth & C.P. Bouche	0.64	0.47	
S. tampicense Dunal			

[a]Samples screened by indirect ELSIA. Virus-free control plants in each treatment remained asymptomatic and healthy.

[b]Host reactions: + = positive reading by ELISA, blank space = negative reading. LHR = localized hypersensitive response, SHR = systemic hypersensitive response, M = mosaic symptoms, NS = no symptoms.

Solanum americanum, S. anguivi, S. gilo, S. nodifolium, S. pseudocapsicum, and *S. sessilifolium* produces localized HR. The presence of TMGMV infection is detected by indirect ELISA in *S. macrocarpon, S. nigrum, S. rostratum,* and *S. spinosissimum*. Of these, *S. nigrum* and *S. rostratum* do not develop visible symptoms, while *S. macrocarpon* and *S. spinosissimum* develop mosaic symptoms. The cultivated pepper species *Capsicum annuum* (California Wonder and Jalapeño) develops systemic HR. In the first trial, two California Wonder (bell pepper) plants were killed within 2 to 3 weeks after inoculation. In the second trial, one jalapeño pepper plant was killed. The remaining *C. annuum* plants have necrotic lesions on leaves and stems, minor leaf distortion, fruit malformation, and stunting. *Lycopersion esculentum* cv. Better Boy (tomato) and *Solanum melongena (*eggplant), as well as the remaining *Solanum* species, are immune and therefore nonhosts to TMGMV. This is confirmed by the indirect ELISA absorbance values for leaf extracts (antigen samples) from corresponding inoculated and control plants (**Table 4**).

Field Trials of Efficacy of TMGMV as a Herbicide for TSA

In a first set of field trials, the inoculum is prepared by triturating up to 10.0 g vacuum-dried, TMGMV-infected Turkish Samsun *nn* tobacco leaf tissue (preferably 0.5, 1.0, 1.5, and 3.0 g) in 10-20 ml of sodium phosphate buffer (pH 7.2). The extracted samples are then filtered by means known in the art, for example, strained through sterile cheesecloth into capped vials. At the time of inoculation, the virus-buffer mixture is poured into 1 liter of sterile deionized water. One gram of carborundum (320 grit) was added to each liter to serve as an abrasive. To prevent contamination of the controls, the virus-free control treatments are applied first followed by the virus treatments.

The efficacy of TMGMV as a bioherbicide for TSA is established through field trials. These trials are conducted at two sites. Field Site No. 1 is in a 5-ha cattle pasture near Hawthorne, Florida, which has a moderate density of TSA infestation. The TSA plants in this field range in maturities from small seedlings to large-canopied, fruit-bearing plants. The trial is performed two times at this site.

specific interaction between a receptor domain of the *R* gene product and a specific pathogen elicitor, a product of the avirulence gene. Different forms of the elicitor can interact differently with the resistance gene. Strong elicitors induce the resistance response quickly, with the pathogen then being confined to a small area surrounding the initial infection site. Weak elicitors slowly induce the hypersensitive response, allowing the pathogen to spread further before it is confined, if at all. These differences are probably due to the availability or affinity of the elicitor to the receptor. Thus, viral elicitors appear to be any viral product that the plant can recognize in order to mobilize a resistance response (Dawson, 1999; Chisholm *et al.*, 2000). Different plants recognize the same viral gene product in a different manner. Conversely, viruses evolve by generating viral gene products that escape host recognition and thus avoid the hypersensitive response that limits their ability to systemically infect the host (Dawson, 1999).

All tobamoviral gene products have been shown to act as an elicitor in some plant. The tobamovirus coat protein was the first viral gene product recognized as an elicitor of an hypersensitive response in tobacco containing the *N* gene, (Saito *et al.*, 1987). The movement protein gene product has been mapped as the elicitor of the hypersensitive response in tomato containing *TM-2* and *TM-2*^{2} genes, (Weber and Pfitzner, 1998). *N*-gene-mediated hypersensitive response in tobacco is induced by the tobamovirus replicase. (Padgett *et al.*, 1997).

Several techniques have proven highly effective in identifying viral factors responsible for elicitation of the hypersensitive response. The first is the production of chimeric viruses consisting of genomic segments from different viruses. This method is particularly useful in systems with closely related viruses that have distinct host resistance phenotypes. In many systems, however, both resistance inducing and/or noninducing viruses do not exist. To overcome this problem, heterologous viral vectors can be produced and used for the expression of specific viral components in attempts to assign avirulence functions (Culver, 1997; Shivprasad *et al.*, 1999).

The induction of the hypersensitive response in plants does not necessarily require the presence of the pathogen responsible for the elicitor. Culver and Dawson (1991)

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,494,955 B2

APPLICATION NO.: 10/755,008

DATED : February 24, 2009

INVENTORS : Raghavan Charudattan, Matthew Scott Pettersen, Ernest Hiebert

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

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